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BLOOD AND THE IDENTIFICATION OF BACTERIAL SPECIES

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BY

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Appendix—Reviews of Books.

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BLOOD AND THE IDENTIFICATION OF BACTERIAL SPECIES.

THE identification of any particular bacillus may be either a very easy or a very difficult matter. The detection of tubercle bacilli in sputum can be pretty safely entrusted to any conscientious medical student who has done his course of practical pathology: the determination of typhoid bacilli in the excreta of a patient may require all the resources of an experienced bacteriologist. During the last two years, however, the development and proper understanding of previous isolated or misinterpreted observations has given us a new and generally trustworthy method of recognising many species of micro-organisms. And more than this, by a simple reversal of the procedure it has been possible to supply a new means and aid to clinical diagnosis—another addition to practical medicine derived from experimental science.

So for back as 1889 Charrin and Roger (3) demonstrated that the growth of *B. pyocyaneus* (and of other bateria) differed when made in the serum of animals immunised against *B. pyocyaneus* and when made in the serum of normal animals. The differences consisted in (i.) serum remaining more transparent, (ii.) formation of granular agglomerations and (iii.) microscopically, the formation of chains of altered bacteria, in the former mode of cultivation.

In 1891, Metschnikoff, whilst investigating *Vibrio Metschnikovi*, saw the same phenomena, and noted them as facts requiring further investigation (10). But he made no progress in this direction, being discouraged by failure to obtain the reactions with the bacillus of hog cholera.

In 1893 Issaeff made similar observations with Pneumococci, and later in conjunction with Ivanoff, with *Vibrio Ivanovi.* They attributed the effects obtained to the bactericidal and growth-hindering substances in the serum (8).

In 1895, Washbourn saw similar appearances, and remarked that the mode of growth in the serum seemed to give a good indication of its protective power (13).

All these observers appear to have worked with undiluted serum only, and that too acting for a considerable time. Although they examined the deposit with the microscope they do not appear to have investigated microscopically or otherwise, either the immediate effect of the serum on the bacilli, or the effect of diluting the serum.

In 1894 R. Pfeiffer published what is now known as Pfeiffer's reaction (11), but it was Bordet who, in 1895, trying to simplify Pfeiffer's reaction, first described any observations in which the above-mentioned neglected aspects of the question were considered. He saw the agglomeration and loss of movement, but failed to attribute any specific importance to the reaction (2*a*).

It was therefore left to Durham and Gruber, who had already been more than six months at work on the subject when Bordet's investigations were published, to show how valuable and characteristic this reaction could be made, and to establish its general applicability (4*a*, 6*a*).

In March, 1896, I applied the reaction to the diagnosis of enteric fever in man, but scarcity of material and other delays prevented any paper on the subject appearing before September (7*a*). Meanwhile Widal, profiting by a hint thrown out by Professor Gruber at the Wiesbaden Congress (6*c*) in April, and having more cases at his disposal, was able to publish a communication on serum-diagnosis early in July[1] (14*a*).

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[1] Professor Widal has persistently ignored the dates given in my articles and constantly represents me as having merely confirmed his results. As a matter of fact, so far as I am concerned, the invention of serum diagnosis was made independently, possibly earlier, and arrived at from an opposite point of view. On the other hand, there are certain points, *e.g.*, (i.) action of serum from other than enterica patients, (ii.) action of human serum on bacilli other than *B. typhosus*, (iii.) variability of strength of serum in enterica patients from day to day, (iv.) difference of sera of mother and child, and (v.) desirability of accurate dilution, contained in my papers; these Professor Widal has incorporated and repeated without acknowledging their origin.

Pfeiffer's reaction is observed by injecting a mixture of cholera vibrios together with a small quantity of serum from an animal immunised against cholera into the peritoneal cavity of a normal animal. By withdrawing drops of fluid from the abdomen at different intervals it can be seen that the micro-organisms first lose their motility, then become transformed into spherules and finally disappear. The rapidity and completeness of the reaction depends on various factors (*e.g.*, potency of serum, extent of dilution, etc.). It is not so easily performed with typhoid bacilli.

Pfeiffer thought vital action essential, but Bordet found that the same phenomena could be observed outside the body if the temperature were kept at 37°C. He generally performed the reaction with a mixture of bacilli, "immune"[1] and normal serum, but he reccgnised that it could occur well with only "immune" serum, and also, although generally more feebly with only normal serum from various sources. Bordet laid chief stress on the granular degeneration of the micro-organisms rather than on the agglomeration into groups.

Durham and Gruber examined the phenomena much more thoroughly, and extended it to several kinds of micro-organisms. They found that the addition of a small quantity of "immune" serum to an emulsion of the corresponding bacillus in a test tube caused a precipitation of the bacilli in the form of fine granules, the supernatant liquid becoming clear. In fact the reaction resembled an ordinary chemical reaction occurring slowly. A control tube containing only bacilli would remain uniformly turbid. The rapidity and completeness of the reaction were found to depend on the same causes as in Pfeiffer's reaction. If the same experiment is performed with smaller quantities so that the process can be observed microscopically, under suitable conditions the following stages are seen. The bacilli at first isolated and moving rapidly about and across the field gradually slow down and begin to hang on one to another; groups of three or four are thus formed which then adhere to other groups, until, if the reaction be complete, all the micro-organisms are collected into large clumps, with total loss of movement. Should the serum be very potent and not sufficiently diluted, agglomeration and loss of movement may occur instantly, so that by the time the slide is brought under the microscope the reaction is already completed. On the other hand, with a weak or much diluted serum, the phenomena may remain permanently incomplete, so that several isolated micro-organisms are still seen, and even some of those in the clumps may retain their motility. So delicate, however, is the reaction that serum from a very highly immunised animal will produce an effect on its corresponding bacillus even when diluted 500,000 times. The action is to a considerable degree specific (or as Durham prefers to term it, special), for a "cholera" serum will not act on typhoid bacilli, and "typhoid" serum will not act on coli bacilli. On the other hand, "cholera" serum will affect to some extent certain vibrios which are not cholera, and "typhoid" serum will agglomerate *B. enteriditis.* But there is generally some difference, and any given serum acts best, *viz.*, in greater dilution, on its own kind of bacillus, so that it can be used for its identification, without causing confusion.

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[1] For the sake of brevity, "immune," "typhoid," "cholera," etc., serum will be used for the serum of an immunised animal, for the serum of an animal immunised against cholera, typhoid, etc., respectively.

The extent to which any serum can be diluted and yet show an effect depends in part on the virulence of the culture employed; the more attenuated the culture, the weaker the serum solution necessary to produce an effect. It is to this grouping power that Durham gave the name "clumping action" of the serum, and the hypothetical body producing it was termed by Gruber "agglutinin".

It is obvious that the reaction can be applied to the identification of various kinds of bacilli, if we possess specimens of the corresponding "immune" sera; and perhaps, as will be indicated later, it may be of use in the discovery of new forms. In spite, therefore, of the sources of error just mentioned, a "typhoid" or "cholera" serum can prove of great use in determining whether doubtful organisms belong to these species. It is also clear that the reverse will be true: that by means of a known bacillus we can identify an unknown serum, and can with comparative ease ascertain whether an animal is at all, or to what extent, protected.

That the "agglutinin" is distinct from the bactericidal and paralysing substances in "immune" or other serum appears from the following considerations. An "immune" serum exhibiting strong agglutinative power need not be appreciably more bactericidal than normal serum. A normal serum may exhibit agglutinative without paralysing action (7*a*). Agglutinins are not produced in the serum of animals immunised against certain micro-organisms; *e.g.*, *B. diphtheriæ* and certain micrococci.

Motility of the organism is not, as was at first supposed, essential for agglomeration. Not only can agglutination occur using dead, motionless bacilli instead of active living ones (2*b*), but it may be seen also with some non-motile bacteria and cocci. With some cocci I have seen a phenomenon resembling the loss of movement of motile bacteria. Before the addition of the serum, the cocci usually exhibit a Brownian movement which is much restrained or even entirely inhibited by the action of suitable serum (7*b*).

Gruber explains the phenomenon of agglomeration by the supposition that the agglutinin causes the enclosing membranes of the bacilli to swell out and become sticky, and that they consequently adhere to each other when they come in contact. He also put forward the idea that the effect of the special agglutinin is thus to facilitate the action of the general bactericidal substances common to all sera; and his theory of immunity is based on these two fundamental ideas.

The theory has not been unanimously accepted. Pfeiffer considers each serum to have a strictly specific action. Salimbeni does not believe agglutination to occur inside the body (12). Nobody has been able as yet to demonstrate a swelling of the capsule. Be all this as it may, the observed facts, without any theory, form a valuable addition to bacterial knowledge.

The "active" immunisation of animals is usually attained by the injection, either hypodermically or into the peritoneal cavity, of dead or attenuated cultures of the micro-organisms. The destruction of the culture used may be effected by the use of either heat or disinfectants, but too great an application of either diminishes or destroys the immunising power of the injection (14*b*). The date on which the reaction first appears after injection is rather variable, and depends on two or three factors, although chiefly on the strength of culture. Thus a guinea-pig which I injected with living cholera culture on 18th April, 1896, showed the reaction well on the 21st, whilst one injected with dead cholera culture on 16th March, 1896, did not exhibit marked agglutinative action till 23rd March. It is not even necessary to inject the bodies of the bacteria: filtered cultures give similar but weaker or more delayed results (14*b*, 9). By the simultaneous injection of two kinds of micro-organisms agglutinins for both can be produced in the same serum (14*b*).

"Passive" immunisation is generally produced by the injection of serum from an actively immunised animal. In such a case the agglutinative property shows itself much sooner. Thus, in a guinea-pig which I injected at 3 P.M. on 9th March, 1896, agglutinin could be detected in the blood already at 3·30 P.M., and had attained its maximum strength about 6·50 P.M., remaining at the same strength for about one week afterwards. An actively immunised guinea-pig generally possesses agglutinative serum for a considerably longer period.

The nature of the agglutinating substance has not yet been distinctly ascertained. Filtration of the serum through a porcelain filter diminishes its power. Precipitation by neutral salts of the globulin of the serum or the fibrinogen of the plasma causes loss, or sometimes only diminution, of agglutinative power in the filtered fluid. Similarly serum is generally only two-thirds as powerful as plasma, so that the agglutinin seems to be in some way bound up with the proteids of the blood. Leucocytes, when shed, do not apparently give off any agglutinin. Temperature affects agglutinin in proportion to its height and the time it is allowed to act. Ten minutes at 75° C. destroys the substance, and an hour at 57° C. causes a diminution of effect. Merely heating to 60° C. does not deprive serum of its power (1, 14*b*).

What has been said hitherto relates nearly entirely to animals, and, of these, to guinea-pigs. We shall now consider the question mainly in relation to man.

The serum of normal guinea-pigs rarely exhibits agglutinative power, but that of some other animals, particularly of the horse, sometimes show it in a marked but varying degree. So, too, does the serum of man. The blood of many individuals possesses a pronounced agglomerating action on several forms of micro-organisms; but the quantity of each agglutinin is small, not showing itself when the serum is diluted more than eight times (7*a*). Thus normal human serum presents some striking differences from that of immunised guinea-pigs, principally in its multiple action. We shall consider later whether this is due to the presence of a common agglutinin or small quantities of each special agglutinin.

When normal serum has any agglomerating action it is often nearly equal on at least three kinds of bacteria—*B. typhosus*, the cholera vibro and *B. coli communis*—and its action on each disappears at about the same degree of dilution. In one diseased condition marked by the evident presence of abnormal substances in the serum, namely jaundice, the agglutinating action is always present; and if the serum be not diluted, it is as rapid and complete as with the serum of an immunised animal. Neither normal nor icteric serum, however, possesses the power of inhibiting movement. For this reason it seems probable that during the process of immunising there are formed in the specific sera two substances, one to cause the agglomeration and another to inhibit movement, for this latter quality is nearly as characteristic of such sera as the former.

If this agglutinative property carry with it a proportionate amount of immunity then a considerable number of individuals are slightly immunised against typhoid, against cholera, and probably also against other diseases.

It cannot yet be considered settled whether this property is inherited or acquired. Experiments on animals seem to show possibility of inheritance (14 *b*), but if in these experiments the inoculations were made into the peritoneal cavity there is an obvious source of error. In the few human cases which I have been able to examine, the blood of the child was usually inactive although that of the mother was generally, sometimes even powerfully, active. In one instance where the mother had had enterica five years previously, the child's serum exhibited but very feeble agglutinative power. In one case where the child was born during an attack of enterica in the mother the serum was very active, but in another it was not so; possibly in the first case the child was also passing through an attack. If the specific agglutinins are inherited they should be found fairly frequently in the sera of new-born children, especially abroad, where a larger percentage of the population have had either enterica or cholera. According to my own observations, in Austria at any rate, a greater percentage of adult than infant sera possesses this property, tending to show that it is an acquired quality. It may be that a more extended series of observations would reverse these figures. Anyway, in light of these differences of hæmic properties, statistics on the relative immunity (if it exist) of the children of "typhoid" parents would be of interest.

It was natural to suppose, as the result of experiments on animals, that typhoid (or cholera) patients, who were thus passing through a natural immunising process, would produce agglutinins in their blood. Observation showed this supposition to be correct, and vaccination experiments on man have confirmed it. In the latter case agglutinins have been observed in the blood already on the second day after injection (15*b*). When the infection of typhoid takes place in the natural way owing to the smallness of the initial dose, the agglutinin is not generally found in the serum until two or three days after the nominal commencement of illness, *viz.*, about sixteen days after infection. This may be taken to be about the time when the contest between organism and micro-organism seriously begins. In cholera, with its much shorter incubation period and rapid development, we should expect to, and actually do, find the reaction earlier.

If the normal agglutinin in the blood is of a non-specific character, then an attack of enterica produces an entirely new substance and does not add to what is already present, because the effect of the serum on cholera vibrios and coli bacilli remains the same, only that on typhoid bacilli is increased.

The rate of agglutinin production varies in different cases as might be expected, and there is also a remarkable variation from day to day in the agglutinative power of the serum. These variations can hardly be entirely due to experimental errors. In immunising animals I have often noticed that the agglutinative power seemed to increase irregularly. Moreover, it is known that the agglutinin is used up during its action on the bacilli, this being one reason for always employing a constant quantity of culture. Consequently, it appears possible that a rapid increase in the number of micro-organisms, which may easily occur if they light on some fresh suitable soil, would use up the available agglutinin and reduce the amount temporarily below what it had been the day before and may reduce it below the level of normal blood. And it explains why the most severe cases of typhoid may have comparatively little agglutinin in their blood.

By the aid, therefore, of a known culture of typhoid bacilli and by suitable dilution of the serum to be tested to avoid any error due to the presence of normal agglutinin, it is possible to diagnose enterica by the agglutinin contained in the patient's blood. It is obvious that the principles of serum-diagnosis apply to other diseases as well as enterica. The method has indeed been used for the diagnosis of cholera, Malta fever and glanders. In diphtheria and tubercle it has not yet been successful. It might very well be applied also to the diagnosis of pneumonia, erysipelas, appendicitis and influenza if required, since it is not necessary that the specific organism should be motile. From a case of scarlet fever I isolated a coccus which agglutinates with scarlet fever serum (*7b*).

One general precaution must be mentioned regarding the serum-diagnosis of any disease. Although absurdly obvious, neglect of it has already led into error; namely, that it is no good applying the test if injections of "antitoxic" serum have been made and agglutinins thus artificially introduced.

Not only may the agglutinin reaction be applied to the diagnosis of disease already present, but is also likely to be of use in the discovery of specific micro-organisms as yet unknown, *e.g.*, small-pox, typhus, measles, etc. For this purpose bacteria must be isolated from the various tissues and their reaction with the serum of patients convalescent from the disease in question ascertained. Naturally, any positive result must be controlled with other sera, and by suitable dilution of the specific serum.

There is one source of fallacy against which it is necessary to guard. Some infectious diseases, *e.g.*, scarlet fever, and small-pox in its later stages, are nearly certainly cases of mixed infection, more particularly with the organisms of sepsis. Under such conditions more than micro-organism might give a reaction with the serum, and other criteria would have to decide which is the specific one. Partly for this reason I do not consider the coccus above mentioned to be the specific organism of scarlet fever.

Without Durham and Gruber's exact and complete investigation and extension of the older isolated observations, a valuable addition to both practical bacteriology and to practical medicine would have been, if nothing else, for some time delayed. The application of the serum test to disease in man is but the natural outcome of their work. Being now part of routine diagnosis it is past the stage when the name of any individual should be attached to it, but in the history of this new departure in diagnosis their names have the first claim to be mentioned.

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